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A survey on the milk chemical and microbiological quality in dairy donkey farms located in NorthWestern Italy

Laura Cavallarin [a], Marzia Giribaldi [a, *], Maria de los Dolores Soto-Del Rio [b], Emanuela Valle [b], Gandolfo Barbarino [c], Maria Silvia Gennero [d], Tiziana Civera [b]

[a] Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche, Largo Braccini 2, 10095 Grugliasco, Italy
[b] Dipartimento di Scienze Veterinarie, Università di Torino, Largo Braccini 2, 10095 Grugliasco, Italy
[c] Regione Piemonte, Direzione Sanità, Settore Prevenzione e Veterinaria, Via Lagrange 24, 10123 Torino, Italy
[d] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Torino, Italy

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ABSTRACT

There is a growing interest in donkey's milk as food for sensitive consumers, such as infants with cow's milk protein allergy and elderly people. The aim of this study was to carry out a survey on the dairy donkeys farming in Piedmont, Italy. The research was conducted in order to analyze the farm characteristics as well as the chemical and microbiological quality of milk. All the farms were small-sized, family-run, and, in most cases, animals were farmed semi-extensively. The donkey milk from Piedmont farms was characterized by a protein content around 1.5 g/100 mL and a fat content lower than 0.1 g/100 mL. Lysozyme activity was considerably higher than that reported in raw cow milk. The milk microbiological profile greatly differed among the farms. Milk sampled in the farm that performed hand milking showed total viable counts significantly lower than milk collected in the farms equipped with automatic milking. Samples were tested for several pathogens and negative results were observed, except for the detection of *Bacillus cereus* in one sample. The survey provided useful data for the laying down of recent regional regulation for the production and commercialization of donkey's milk. The results of the survey indicate that further research is needed in order to define the best management and nutritional strategies for the improvement of the quali-quantitative production of dairy donkeys.

1. Introduction

There is a growing interest in donkey farming for milk production, since donkey's milk (DM) is considered a valid alternative foodstuff in terms of clinical tolerability, palatability and nutritional adequacy for children affected by cow's milk protein allergy (Iacono et al., 1992; Mansueto et al., 2013; Monti et al., 2007, 2012).

Since 2005, the number of published papers on DM has increased to almost 30 per year, the great majority by Italian researchers. Although data covering the potential of DM use as a substitute of cow's milk for allergic individuals are available, information regarding donkey farm management and some aspects of milk production are limited, such as the study of its main microbial communities. The majority of surveys on DM quality were carried out in the South of Italy, mainly on Martina Franca (D'Alessandro, De Petro, Claps, Pizzillo, & Martemucci, 2009; D'Alessandro & Martemucci, 2007; D'Alessandro, Martemucci, Jirillo, & Leo, 2011; Martemucci & D'Alessandro, 2012) and Ragusana breeds (Alabiso, Giosuè, Alicata, Mazza, & Iannolino, 2009; Giosuè, Alabiso, Russo, Alicata, & Torrisi, 2008). One very recent report was published on Amiata donkey milk chemical composition (Martini, Altomonte, & Salari, 2014). Some data are also available for Chinese donkeys (Guo et al., 2007) and for Balkan Littoral-Dinaric breed (Ivanković et al., 2009; Šarić et al., 2012). The literature indicates that fat content in DM ranges from 0.3 to 1.8 g/100 mL, while the protein content is reported to be less variable, with values from 1.4 to 1.8 g/100 mL (Medhammar et al., 2012). DM is also known to contain high concentration of lysozyme, if compared to other mammalian milks. Donkey's lysozyme, similarly to cow's, displays antibacterial activity against a vast number of

Abbreviations: DM, donkey milk; LAB, lactic acid bacteria; PVDF, polyvinylidene fluoride; TVC, total viable count.

* Corresponding author. Tel.: +39 011 670 9231; fax: +39 011 670 9297.
E-mail addresses: laura.cavallarin@ispa.cnr.it (L. Cavallarin), marzia.giribaldi@ispa.cnr.it (M. Giribaldi), mariadelosdolores.sotodelrio@unito.it (M.D. Soto-Del Rio), gandolfo.barbarino@regione.piemonte.it (G. Barbarino), mariasilvia.gennero@izsto.it (M.S. Gennero), tiziana.civera@unito.it (T. Civera).

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bacteria (Conte, Foti, Malvisi, Giacopello, & Piccinini, 2012; Šarić et al., 2012; Zhang, Zhao, Jiang, Dong, & Ren, 2008), affecting directly the composition of the milk microbiome (Sarno, Santoro, Di Palo, & Costanzo, 2012). Very recently, it has been shown that the addition of jenny milk during cheese-making reduced the number of coliforms in the resulting cheese (Cosentino, Paolino, Freschi, & Calluso, 2013). Further compositional aspects of DM, that are not considered in the present study, were extensively reviewed in two recent reports by Salimei and Fantuz (2012) and Medhammar et al. (2012).

Low bacterial counts are generally reported in DM, in comparison to milk and dairy products from other animals (Pilla, Daprà, Zecconi, & Piccinini, 2010; Salimei et al., 2004; Sarno et al., 2012). Previous reports have shown that the DM microbiota is mainly composed by lactic acid bacteria (Šarić et al., 2012; Zhang et al., 2008). Nevertheless, undesirable species bacteria, such as *Streptococcus* spp., *Staphylococcus* spp. and coliforms, have been also detected in DM (Pilla et al., 2010; Sarno et al., 2012).

The aim of this research is to provide the first overview of the chemical and microbiological characteristics of milk produced in dairy donkey farms in the North West of Italy. This outline provided a significant contribution to the recent publication of a regional regulation for the production and commercialization of DM (Regione Piemonte Direzione Sanità Settore Prevenzione e Veterinaria, 2013).

2. Materials and methods

2.1. Farm description and milk sampling

Five donkey dairy farms located in the North Western Italy, in Piedmont, were surveyed during this study, and a questionnaire was filled in for each farm. Data and samples were collected during Autumn 2012 and Spring 2013. The on-farm survey included a face-to-face interview with the farm manager. We developed a questionnaire, which consisted of semi-closed questions, including topics related to farm management and husbandry practices.

The DM samples were obtained from a different number of jennies and by different milking practices, depending on farm management. In each surveyed farm, 1 L of DM was sampled for chemical and biochemical analyses, while six replicate DM aliquots (300 mL each) were collected in sterile bottles for microbiological screening.

Immediately after collection, DM samples were transported, refrigerated and either used fresh (as for microbiological analyses), stored within 6 h from the collection at −20 °C, or lyophilized.

2.2. Milk chemical and biochemical analyses

The DM samples were analyzed for gross energy, dry matter, crude protein and ether extract contents. In addition, the lysozyme activity of each sample was determined using a fluorescence-based assay (EnzCheck® Lysozyme Assay Kit, Life Technologies Italia, Monza, Italy).

Dry matter content was measured on DM using a gravimetric method (Baldini et al., 1996). Gross energy content was determined in excess oxygen by adiabatic bomb calorimeter (Mod. 700, IKA GmbH & Co., Staufen, Germany), using benzoic acid as a reference (26.454 MJ/kg). The nitrogen content in milk samples was estimated by Kjeldahl-based block digestion method (AOAC Official Method 991.20, 2000), using a 2020 Tecator Digestor (VWR International Pbi, Milano, Italy), a Kjeltec-System 1002 Distilling Unit (Foss Italia, Padova, Italy) and a 655 Dosimat automatic titrimeter (Metrohm Italiana, Origgio, Italy). The total fat content of DM samples was measured gravimetrically on ether extract using a Soxhtraction device (VWR International Pbi), following manufacturer's instructions. All the analyses were performed on lyophilized DM samples in triplicate.

Lysozyme activity assay was conducted by EnzCheck® Lysozyme Assay Kit following manufacturer's instruction in triplicate on 500-fold diluted DM samples, using a fluorescence microplate reader (Victor 3D, Perkin Elmer, Waltham, USA), equipped with fluorescein filters (485 nm emission and 535 nm excitation).

2.3. Milk protein profile

DM samples were accurately mixed and vortexed at room temperature in order to ensure homogeneity, then 100 μL from each sample were taken up to 1 mL with milliQ water and vortexed thoroughly again. One aliquot (100 μL) of each diluted sample was mixed with the same volume of NuPAGE® LDS Sample Buffer (2X diluted) (Life Technologies Italia), containing 50 mM dithiothreitol (Sigma Aldrich, Milano, Italy) as reducing agent, and warmed to 70 °C for 10 min. Each sample solution (10 μL) was then loaded onto a 10-well, NuPAGE® Bis–Tris mini gels (12% polyacrylamide, 1 mm width). One lane on each gel was loaded with 5 μL of Mark12® Unstained Standard (Life Technologies Italia).

The proteins were separated on a Novex Mini-cell (Life Technologies Italia) filled with cold 1X NuPAGE® MES SDS Running Buffer (Life Technologies Italia), at 200 V. The gels were then stained with colloidal Coomassie Blue staining (Candiano et al., 2004) and digitized with an ImageScanner device (Amersham Pharmacia, now GE Healthcare Life Sciences, Uppsala, Sweden) at 300 dpi.

Two bands were excised with a sterile scalpel and passively eluted from the gel pieces onto PVDF membranes, as previously described (Reuter et al., 2005) to be subjected to N-terminal sequencing. The membranes were then microsequenced on a Procise 492 protein sequencer (Applied Biosystems, now Life Technologies Italia). All the chemicals used in the procedure were from Life Technologies Italia. The N-terminal amino acid sequences were searched with the MS-Homology software package on NCBI non-redundant database (NCBInr2013.6.17).

2.4. Milk microbiological analyses

For this part of the study, a third sampling was carried out during the 2014 Spring season.

Two aliquots of each DM sample were collected and stored in sterile conditions at 4 °C. One of them was analyzed the day after milking, while the second one was examined 5 days after the sampling.

The total viable count (TVC) was performed on plate count agar, using the inclusion method. One mL of undiluted samples and of each 10^{-1}, 10^{-2} and 10^{-3} dilutions in a mixture of physiological saline solution and peptone (85:15 v:v) (OXOID LTD, Basingstoke, Hampshire, England) were included in the plate count agar (OXOID LTD)and plates were incubated for 72 h at 30 °C before counting. The results were then expressed as cfu/mL (UNI EN ISO 4833:2004). Lactic acid bacteria (LAB) colonies, grown on MRS agar (OXOID LTD) at 30 °C for 48 h in anaerobiosis were counted (ISO/FDIS 15214:1998). According to ISO 6579:2002, for the detection of *Salmonella* spp., BPW enrichment media (OXOID LTD) was used for an incubation of 24 h at 37 °C, a second incubation was performed with Rappaport media (OXOID LTD) (41 °C for 24 h) and with MKTTn (OXOID LTD) at 37 °C for 24 h. Both incubation products were finally grown on XLD agar medium (OXOID LTD) for 24 h at 37 °C.

For *E. coli*, EN/ISO 16649/2:2001 regulation parameters were followed, growing in TBX agar (OXOID LTD) at 44 °C for 24 h.